

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
6 October 2005 (06.10.2005)

PCT

(10) International Publication Number
WO 2005/093100 A1

(51) International Patent Classification⁷: **C12Q 1/68**, 1/68

(21) International Application Number:
PCT/IB2005/000763

(22) International Filing Date: 24 March 2005 (24.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0406864.9 26 March 2004 (26.03.2004) GB

(71) Applicant (for all designated States except US): **QIAGEN GMBH** [DE/DE]; Qiagen Str 1, 40724 Hilden (DE).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **LARSEN, Frank** [NO/NO]; Gaustadveien 93, N-0372 Oslo (NO).

(74) Common Representative: **QIAGEN AS**; c/o Page White & Farrer, 54 Doughty Street, London WC1N 2LS (GB).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM,

AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

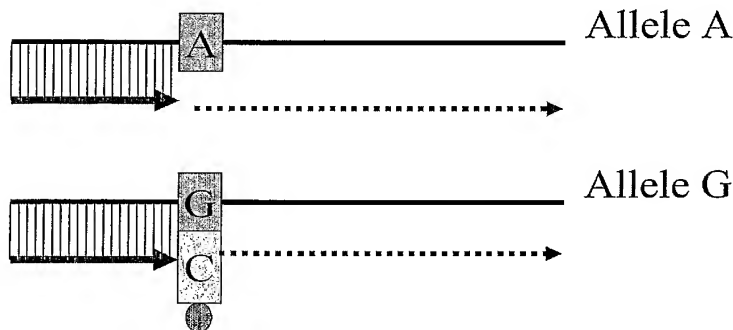
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **NUCLEIC ACID SEQUENCING**



(57) **Abstract:** A method for determining a target nucleic acid sequence, wherein the target nucleic acid sequence is comprised in a preparation comprising a non-target nucleic acid sequence, the target nucleic acid sequence and the non-target nucleic acid sequence each having a first region of common sequence upstream of a first region of dissimilar sequence upstream of a second region of dissimilar sequence, the method comprising: (a) contacting the preparation with an oligonucleotide primer complementary to at least a portion of the first region of common sequence, under conditions to hybridise the primer thereto; (b) contacting the preparation with a first labelled nucleotide bearing a first label, wherein the first labelled nucleotide is complementary to a first template nucleotide comprised in the first region of dissimilar sequence of either the target nucleic acid sequence or the non-target nucleic acid sequence, under conditions to incorporate the first labelled nucleotide either into the primer hybridised to the target nucleic acid sequence or into the primer hybridised to the non-target nucleic acid sequence but not into both; (c) subjecting the preparation to a sequencing reaction, thereby extending the primer to form one or more first-labelled sequencing products comprising the first labelled nucleotide and one or more non-first-labelled sequencing products comprising no first labelled nucleotide; and (d) determining at least a portion of the sequence of the first-labelled sequencing products and/or the non-first-labelled sequencing products, thereby determining at least the second region of dissimilar sequence of the target nucleic acid sequence.



WO 2005/093100 A1